

SEROLOGY

- A. **DON'T'S** in submission of serum samples and tissue specimens. Experience in the past has shown the following problems associated with shipping serum samples or tissue specimens to the laboratory (in order of decreasing occurrence):
1. Bacterial contamination.
 2. Serum not separated from clots.
 3. Chemical (detergent, disinfectants, etc.) contamination of serum causing toxicity to tissue culture or otherwise adversely affecting desired tests.
 4. Hemolysis.
 5. Insufficient quantity.
 6. Overheating or freezing of blood before serum extraction.
 7. Leaky stoppers.
 8. Broken containers.
 9. Improper labeling.
 10. Rotten tissue specimens.
- B. **DO'S** in submission of serum samples and tissue specimens:
The following requirements will help prevent the above listed problems from occurring.
1. Serum
 - a) Collect blood aseptically into a sterile dry tube, refrigerate immediately, allow to clot, centrifuge, and transfer serum aseptically into a second tube.
 - b) Serum must be fresh, clear, unhemolyzed, and uncontaminated.
 - c) Red top Vacutainers[®], B-D, or other non-EDTA/heparin sterile commercially available tubes are ideal.
 2. **Label each tube** (not stopper) with **tube number** and **vet code**. Be careful that writing will be legible upon arrival (use an indelible marker). Identify specimen in a way that will avoid confusion when results are reported. Avoid using animal names. Keep your own log of sample numbers to avoid duplication and confusion. Put tubes in a box so they will not be lost or broken in the packing.
 3. Serum is preferred to be separated, by centrifugation, from clotted blood prior to submission for testing. Do not let whole blood freeze or expose to direct sunlight or high temperature before decanting serum.
 4. Submit at least 1.0 ml of serum for each test requested. Refrigerate the serum until shipment.
 5. Tissues should be collected aseptically and placed individually in well-labeled whirl-pak bags. Place individual whirl-pak bag into a single air tight container and refrigerate immediately.
 6. **Ear notches**, about 3/8 inch x 3/8 inch in size should be freshly collected in clean sterile tubes without any media or fixative in the tube. They should be shipped with sufficient amount of ice packs.
 7. Swab samples should be kept in appropriate transport media and shipped in an insulated container with ice packs.
 8. Refrigerated samples should be shipped with sufficient cool-pak bags and padding. Ship samples in a leak proof insulated container or in sturdy mailing carton which complies with postal or commercial carrier specifications (Page 10). Submit samples early in the week to avoid holding over the weekend by the carrier.
 9. A complete and legible form(s), (including a complete history), must accompany all serologic submissions. Be certain to indicate vaccination history. "All and Current" is not an appropriate vaccination history.
 10. Diagnostic serology samples must be submitted independently from program serology.
EXAMPLE: Serum for Leptospira, IBR, BVD testing must be submitted separately from serum for Brucellosis or "Bangs" serology.
 11. All regulatory charts must include the submitting veterinarian's signature, vet code, and animal identification.

12. Flock check serology should be based upon a sample size of 20-30 samples per flock. National Poultry Improve Plan (NPIP) testing may require larger number of samples.
13. This laboratory is officially certified to perform agar gel immunodiffusion test for *bluetongue*, *bovine leukosis*, and *equine infectious anemia* (the Coggins test for *EIA*), ELISA for *Johne's*, *pseudorabies* serology that includes serum neutralization, latex agglutination and ELISA, and PCR for avian influenza, exotic Newcastle disease, foot-and-mouth-disease and classical swine fever.
14. Equine Infectious Anemia serology (Coggins test) requires at least two (2) ml of clear, fresh, unhemolyzed serum and takes up to 48 hours to complete. Each tube of serum submitted should be identified with **vet code** and the **tube number** corresponding to the tube number on the submission form. A **mane tag** is to be affixed to each horse tested and **tag number** recorded on the submission form. EIA results will be available after 8:30 A.M. the work day following the receiving of serum sample. If additional tests other than EIA are requested, please, submit two samples. The submission form must be completely and correctly completed and signed by the submitting veterinarian. **The lab cannot make any changes or fill in any missing information. All forms not properly completed will be returned for completion.**
15. **Exports:** The submitting veterinarian is responsible for informing the laboratory of any special requirements (i.e., dilutions, type of test). If this information is not supplied, a delay on the required test result could happen.
For information on test regulations, call:
Federal - USDA, APHIS, VS, Veterinarian in Charge - (615)781-5310
State - Office of the State Veterinarian - (615) 837-5120
The samples must be received 10 to 14 working days before the animals are scheduled to leave for the destination.
16. When calling for results, the laboratory must have the submitting veterinarian's name, the owner's name, the animal's name or ID, the sample number or ID, submitting date and bleeding date exactly as provided on the submission form.
17. Advance notice (7-10 days ahead) should be given (and will be greatly appreciated) when submitting a large number of samples.
18. For serodiagnosis in the individual animal, paired sera are recommended to test for specific antibody: the first sample taken when the animal is initially examined (acute phase serum) and the second sample 2-4 weeks later (convalescent phase serum). A rise in antibody titer between the paired samples is a basis for a specific serologic diagnosis for a particular disease. The paired serum samples should be submitted together in order to obtain a better understanding of the diagnostic significance of titers as they relate to the clinic status of the animal.
19. If only a single serum sample can be obtained, it must be taken from a convalescent animal. An acutely ill animal is virtually devoid of antibody against the particular disease-causing agent.
20. If only acutely ill animals are present, swabs or tissues for isolation or culture of the causative agent should also be submitted.

Key to abbreviations for Serology Test Chart:

AGID - Agar gel Immunodiffusion
 CA - Card Agglutination
 CF - Complement Fixation
 ELISA - Enzyme Linked Immunosorbent Assay
 FA - Fluorescent Antibody (Direct)
 HI - Hemagglutination Inhibition
 IFA - Indirect Fluorescent Antibody
 IHA - Indirect Hemagglutination

LA - Latex Agglutination
 MA - Microagglutination
 NVSL - National Veterinary Services Laboratory
 PCR - Polymerase Chain Reaction
 SN - Serum Neutralization
 SPA - Serum Plate Agglutination
 TA - Tube Agglutination

SEROLOGY TEST CHART

Note: All set up date and timetables are an approximation and are subject to change. Please call with any questions or special requests.

TEST	SPECIMEN	SHIPPING CONDITION	TYPE OF TEST	DAYS SET UP	SPECIAL COMMENTS
<u>Avian</u>					
Avian Influenza	Serum	Refrigerated	AGID	M,Tu,W, Thur	Requires at least 1 ml of serum
	Swab (cloacal, tracheal, oropharyngeal)	Refrigerated	Strip test	M-F	
	Swabs (cloacal, tracheal, oropharyngeal)	Refrigerated	PCR		Special request is required.
Chlamydia	Spleen, liver, lung, air sac, conjunctival swab	Refrigerated	FA	M-F	
Mycoplasma gallisepticum	Serum	Refrigerated	HI SPA	M-F	Requires at least 1 ml of serum.
Mycoplasma synoviae	Serum	Refrigerated	HI SPA	M-F	Requires at least 1 ml of serum
Salmonella pullorum	Serum	Refrigerated	MA	Once a week	
<u>Canine</u>					
Anti-nuclear Antibody	Serum	Refrigerated	IFA	M-F	IgG determination. Samples are tested at 1:10, 1:50, 1:250, 1:1250 dilutions.
Blastomycosis	Serum	Refrigerated	AGID	M-F	
Brucella canis	Serum	Refrigerated	CA	M-F	Samples are screened by the card test. Positives will not be reported until confirmed by the tube test.
		Refrigerated	TA	M,Tu,W	Samples are tested at 1:50, 1:100, 1:200 dilutions. A 1:50 titer is considered as Suspicious. A titer \geq 1:100 is considered Positive.
Calicivirus	Serum	Refrigerated	IFA	M-F	IgG, IgM determination. Samples are tested at 1:10, 1:50, 1:250, 1:1250 dilutions.
Coronavirus	Small intestine	Refrigerated	FA	M-F	
	Serum	Refrigerated	IFA	M-F	IgG, IgM determination. Samples run at 1:10, 1:50, 1:250, 1:1250 dilutions.
Cryptococcus	Serum	Refrigerated	LA	Outsource	Fee \$19.00

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TEST	SPECIMEN	SHIPPING CONDITION	TYPE OF TEST	DAYS SET UP	SPECIAL COMMENTS
Distemper Virus	Lung, kidney, spleen, urinary bladder, brain, stomach, liver, blood smear	Refrigerated	FA	M-F	
	Serum	Refrigerated	IFA	M-F	IgG, IgM determination. Samples run at 1:10, 1:50, 1:250, 1:1250 dilutions.
Ehrlichia canis	Serum	Refrigerated	IFA	M-F	Samples are tested at 1:50 dilution.
Herpesvirus	Lung, liver, kidney, spleen, lymph node	Refrigerated	FA	M-F	
	Serum	Refrigerated	IFA	M-F	IgG, IgM determination. Samples run at 1:10, 1:50, 1:250, 1:1250 dilutions.
Histoplasmosis	Serum	Refrigerated	AGID	M-F	
Infectious Canine Hepatitis	Serum	Refrigerated	IFA	M-F	IgG, IgM determination. Samples run at 1:10, 1:50, 1:250, 1:1250 dilutions.
Lyme disease	Serum	Refrigerated	IFA	M-F	Samples run at 1:64, 1:128, 1:256, 1:512 dilutions.
Neospora caninum	Brain, lung, liver, kidney, muscle, skin	Refrigerated	FA	M-F	
	Serum	Refrigerated	IFA	M-F	Samples are tested at 1:50.
Parvovirus(CPV)	Intestine (jejunum, ileum), spleen, mesenteric lymph node	Refrigerated	FA	M-F	
	Serum	Refrigerated	IFA	M-F	IgG, IgM determination. Samples run at 1:10, 1:50, 1:250, 1:1250 dilutions.
Rocky Mountain Spotted Fever (RMSF)	Serum	Refrigerated	IFA	M-F	Samples run at 1:64, 1:128, 1:256, 1:512 dilutions.
Equine					
Equine Infectious Anemia (EIA)	Serum	Refrigerated	AGID	M-F	Complete EIA form (VS Form 10-11) with submitting veterinarian's signature is required.

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TEST	SPECIMEN	SHIPPING CONDITION	TYPE OF TEST	DAYS SET UP	SPECIAL COMMENTS
Equine Rhinopneumonitis Virus (ERV)	Lung, liver, spleen, fetal tissues	Refrigerated	FA	M-F	
	Serum	Refrigerated	SN	M, Th, F	Samples are tested at dilutions: 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256.
Potomac Horse Fever (PHF)	Serum	Refrigerated	IFA	M-F	Samples are tested at dilutions: 1: 80.
West Nile virus WNV)	Serum	Refrigerated	ELISA	Once a week	Tested at 1:400.
	Cerebrospinal fluid	Refrigerated	ELISA	Once a week	Tested at 1:2.
<u>Feline</u>					
Chlamydia	Conjunctival smear, nasal swab, lung	Refrigerated	FA	M-F	
Feline Calicivirus	Serum	Refrigerated	IFA	M-F	Samples are tested for IgG titers at 1:10, 1:50, 1:250, 1:1250 dilutions.
Feline Herpesvirus	Nasal swab, conjunctival swab, tonsil, trachea, lung	Refrigerated	FA	M-F	
	Serum	Refrigerated	IFA	M-F	Samples are tested for IgG titers at 1:10, 1:50, 1:250, 1:1250 dilutions.
Feline Immunodeficiency Virus (FIV)	Serum	Refrigerated	IFA	M-F	Samples are tested at 1:25 dilutions.
Feline Infectious Peritonitis (FIP)	Affected tissues (liver, spleen, lymph nodes, <i>brain</i>)	Refrigerated	FA	M-F	
	Serum, pleural or peritoneal fluid	Refrigerated	IFA	M-F	Samples are tested at 1:6400 dilution.

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TEST	SPECIMEN	SHIPPING CONDITION	TYPE OF TEST	DAYS SET UP	SPECIAL COMMENTS
Feline Leukemia (FeLV)	Blood smears	Slide mailers	IFA	M-F	Submit at least 2 smears. Thick smears are not acceptable. Do not use old or used slides.
	Serum	Refrigerated	IFA	M-F	Samples are tested at 1:25 dilutions.
Panleukopenia(FPL)	Small intestine, lymph node, spleen, aborted fetus	Refrigerated	FA	M-F	
	Serum	Refrigerated	IFA	M-F	Samples are tested for IgG titers at 1:10, 1:50, 1:250, 1:1250 dilutions.
Toxoplasmosis	Serum	Refrigerated	IHA	M-F	A titer \geq 1:64 is considered positive.
Porcine					
<i>E.coli</i> Pilus	Small intestine, feces	Refrigerated	IFA	M-F	Test for K88, K99, P987 serotypes
Hemagglutinating Encephalomyelitis Virus	Brain	Refrigerated	FA	M-F	
Leptospirosis	Serum	Refrigerated	MA	M-F	Test for 6 serovars - canicola, grippotyphosa, hardjo, icterohemorrhagiae, pomona, and bratislava. Samples are tested at an initial dilution 1:100.
	Kidney	Refrigerated	FA	M-F	
Porcine Parvovirus	Fetal tissues	Refrigerated	FA	M-F	Tested at 1:10, 1:50, 1:250, 1:1250 dilutions. A single serum sample from the dam is of little value since the breeding herd is often seropositive.
	Fetal serum or fetal fluid	Refrigerated	IFA	M-F	
Porcine Respiratory and Reproductive Syndrome (PRRS)	Serum	Refrigerated	IFA	M-F	Samples are tested at 1:20 dilution.

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Pseudorabies	Brain, lung, tonsil, kidney	Refrigerated	FA	M-F	Results are reported as positive or negative. Serum samples must be clear, unhemolyzed. Serum is screened at 1:4 dilution
	Serum	Refrigerated	ELISA-gp1	Special request required M-F	
	Serum	Refrigerated	LA		
	Serum	Refrigerated	SN	M, Tu, W,F	
Rotavirus	Feces, intestine	Refrigerated	ELISA	M-F	
Swine Influenza	Nasal secretions, trachea, lung	Refrigerated	FA	M-F	
Transmissible Gastroenteritis (TGE)	Intestine (jejunum, ileum)	Refrigerated	FA	M-F	
<u>Ruminant</u>					
Anaplasmosis	Serum	Refrigerated	CA	M-F	Samples must be at least 24 hours old from time of collection to time test conducted. Time of collection must be written on submission form. Result is reported as positive or negative. Titers begin at 1:5.
	Serum	Refrigerated	CF	M-F	
	Serum	Refrigerated	ELISA	Once a week	
Bluetongue	Serum	Refrigerated	AGID	M-F	
Bovine Leukosis (BLV)	Serum	Refrigerated	AGID	M,Tu,W,F	

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Bovine Respiratory Syncytial Virus (BRSV)	Lung, bronchial lymph node	Refrigerated	FA	M-F	Samples are tested at 1:50 dilution.
	Serum	Refrigerated	IFA	M-F	
Bovine Viral Diarrhea (BVD)	Lung, intestine, turbinate, trachea, swabs from lesions, fetal organs	Refrigerated	FA	M-F	Tested at dilutions: 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256.
	Serum	Refrigerated	SN	Tu -F	
Bovine Viral Diarrhea Persistent Infection (BVD PI)	Ear notch, serum	Refrigerated	ELISA	Once a week	Ear notches are the samples of choice.
Caprine Arthritis-Encephalitis (CAE) / Ovine Progressive Pneumonia (OPP)	Serum	Refrigerated	AGID	M-F	
Chlamydia	Lymph node, tissues of aborted fetus, joint fluid	Refrigerated	FA	M-F	Specifically request chlamydia.
Clostridium	Gangrenous muscle, liver, lung	Refrigerated	FA	M-F	Test for <i>C. chauvoei</i> , and <i>C. novyi</i> .
Coronavirus	Intestine (spiral colon, ileum, and jejunum)	Refrigerated	FA	M-F	
<i>E.coli</i> Pilus	Small intestine	Refrigerated	IFA	M-F	Test for K99 serotype
Epizootic Hemorrhagic Disease (EHD)	Serum	Refrigerated	AGID	M-F	

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TEST	SPECIMEN	SHIPPING CONDITION	TYPE OF TEST	DAYS SET UP	SPECIAL COMMENTS
Infectious Bovine Rhinotracheitis (IBR)	Lung, trachea, turbinate, aborted fetal tissues	Refrigerated	FA	M-F	
	Serum	Refrigerated	SN	Tu,W,TH,F	Tested at dilutions: 1:4, 1:8, 1:16, 1:32, 1:64, 1:128, 1:256.
Johne's Disease (Mycobacterium paratuberculosis, Mptb)	Serum	Refrigerated	CF	M-F	Titers: 1:8 – Negative, 1:16 – Suspicious, 1:32 – Positive.
	Serum	Refrigerated	ELISA	Once a week	
	Bacterial culture		PCR	Once a week	PCR is used as a confirmatory assay for the growth of Mptb in bacterial cultures.
Leptospirosis	Kidney	Refrigerated	FA	M-F	Test for 6 serovars - canicola, grippityphosa, hardjo, icterohemorrhagiae, pomona, and bratislava. Samples are tested at an initial dilution 1:100.
	Serum	Refrigerated	MA	Twice a week	
Listeria	Serum	Refrigerated	CA	M-F	Test for Type 1 and Type 4 serotypes. Serum is screened at an initial dilution 1:20.
Neospora caninum	Brain, lung, kidney, liver	Refrigerated	FA	M-F	
	Serum	Refrigerated	IFA	M-F	Tested at 1:200 dilution.
Rotavirus	Feces, intestine (spiral colon, ileum, and jejunum)	Refrigerated	ELISA	M-F	
Toxoplasmosis	Serum	Refrigerated	IHA	M-F	A titer \geq 1:64 is considered positive.